

FACTORS INFLUENCING REGENERATION AND POLARITY DETERMINATION IN TUBULARIA CROCEA

ABRAHAM GOLDIN

(From the Department of Zoölogy, Columbia University, the Marine Biological Laboratory, Woods Hole, Mass., and Queens College, N. Y.)

The regeneration of hydroids has been studied extensively, and the subject has recently been reviewed by Barth (1940*b*). Of the environmental factors which effect regeneration in the hydroids, the role of oxygen has been studied in the most detail (Miller, 1937; Barth, 1938*a*; Zwillling, 1939; Rose and Rose, 1941). Several evidences have been presented, however, which indicate that the accumulation of waste products may have an inhibitory effect on regeneration. When the cut ends of *Tubularia* stem segments are covered with glass capillaries, regeneration is inhibited at the covered ends (Barth, 1938*a*). Rose and Rose (1941) showed that accumulated waste products may inhibit regeneration at a cut surface of *Tubularia*, even when a plentiful supply of oxygen is available at the cut surface. Komori (1933) reported that lowered pH may inhibit regeneration. Miller (1939) reversed the normal polarity of *Tubularia* stems by treating the distal ends with a 90 per cent O₂: 10 per cent CO₂ mixture, while the proximal ends were exposed to oxygen.

Attempts to analyze the role of environmental factors in regeneration and the origin of polarity during regeneration are complicated by the heterogeneous nature of the *Tubularia* stem. In the *Tubularia* stem there is a gradient in the rate of regeneration (Barth, 1938*b*), as well as a gradient of oxygen consumption (Barth, 1940*a*). Further complication is introduced by the dominance which distal levels of the stem exert over proximal levels (Barth, 1938*b*). In order to obtain a clear analysis of the role of the environment in regeneration, it is advantageous to work with a homogeneous system. Such a system is provided by the *Tubularia* coenosarc fragments described by Goldin and Barth (1941). When the coenosarc is removed from the perisarc, a series of reorganizational changes occurs involving both morphological and physiological dedifferentiation. A spherical mass of coenosarc cells is formed, in which there is a complete loss of the original polarity of the stem. The polarity relationships which are developed during the regeneration of the coenosarc fragment are therefore new, and have no relation to the original polarity present in the intact stem.

The experiments, using both coenosarc fragments and stem segments, were designed in order to study the effects of oxygen and accumulated waste products in regeneration. They may be described under the following headings: (1) inhibition of regeneration inside of glass caps; (2) determination of polarity by the differential exposure of coenosarc; (3) the extent of acid metabolite accumulation; and (4) the effect of increased hydrogen ion concentration on regeneration. The author wishes to thank Professor L. G. Barth, under whose supervision this work was carried out, and Professor H. B. Steinbach for his helpful suggestions in the preparation of the manuscript.

METHODS EMPLOYED IN THE CAPILLARY EXPERIMENTS

The method of treatment of the coenosarc fragments was essentially the same as that described by Goldin and Barth (1941). Segments of stem ten millimeters long were cut from the region of *Tubularia* stems extending from five to fifteen millimeters proximal to the hydranth. The coenosarc tissues were expressed from the perisarc and were kept on agar (2 per cent agar in sea water) in running filtered sea water, until ready to be used in the experiments. When stem segments were used, they were taken from the same region of the stem (5–15 mm. proximal to the hydranth), but the perisarc was not removed. The capillaries were drawn from clean glass tubing (7 mm. inside diameter) to a diameter of approximately one millimeter. The capillaries were cut into 25 mm. lengths, and when ready for use, were filled with filtered sea water. A capillary was chosen for each coenosarc fragment so that the fragment would fit tightly enough not to be dislodged during the course of the experiment. In order to manipulate the coenosarc fragments without injury to them hair loops were employed. After the coenosarc fragments were inserted into the capillaries, the latter were submerged in running filtered sea water for the duration of the experiment. The capillaries were all kept one half inch below the surface of the water in order to insure uniformity of conditions. The temperature of the sea water during the experiments was $18 \pm 2^\circ \text{C}$. The operations and observations were made with the aid of a binocular microscope.

INHIBITION OF REGENERATION INSIDE OF GLASS CAPS

Barth (1938a) found that regeneration was inhibited when the cut end of a *Tubularia* stem was covered with a glass capillary. This experiment was performed in order to determine whether inhibition could likewise be effected using coenosarc fragments. Dedifferentiated coenosarc

fragments (twenty-four hours after removal from the perisarc) were inserted in capillaries, midway between the two open ends. Stem segments ten millimeters long were placed in the center of similar capillaries. Other coenosarc fragments and stem segments were kept in running filtered sea water as controls.

Regeneration was inhibited in all cases when the coenosarc fragments and stem segments were covered with capillary tubing. Thirty-one coenosarc fragments failed to regenerate one hundred and fifty hours after being placed in the capillaries (174 hours after removal of the coenosarc from the perisarc). Twenty-three out of twenty-five coenosarc fragment controls regenerated ninety hours after removal of the coenosarc from the perisarc. Ten stem segments placed in capillaries showed no evidences of regeneration after one hundred and forty-two hours, while all of the twenty control stem segments regenerated after seventy hours. When coenosarc fragments and stem segments were removed from the capillaries, many of them regenerated, indicating that the inhibition is reversible.

DETERMINATION OF POLARITY BY THE DIFFERENTIAL EXPOSURE OF COENOSARC

Since the covering of a coenosarc fragment by capillary glass tubing is sufficient to cause inhibition, a differential exposure could be accomplished by placing a coenosarc fragment inside the tip of a capillary so that half of the surface is covered by the capillary while the other half is exposed to the sea water in the dish. The coenosarc fragments were placed in the capillaries at time intervals ranging from twenty-four to forty-eight hours after removal from the perisarc. Coenosarc fragments and stem segments were kept in running filtered sea water as controls.

The results of these experiments are summarized in Table I. Seventy-nine coenosarc fragments placed in the tips of the capillaries regenerated new hydranths at the exposed surface of the fragment, while none regenerated at the capped surface. Thus, all regenerants were unipolar, and polarized in the direction of the open end of the capillary. The control coenosarc fragments, on the other hand, gave rise to unipolar, bipolar, bipolar-unipolar, and multipolar regenerants, as described by Goldin and Barth (1941). Since a coenosarc fragment, at twenty-four hours after removal from the perisarc has lost its original polarity relationships (Goldin and Barth, 1941), the polarity of the regenerant is new, and is developed in response to the imposed environmental differential. It may be noted that, although at 48 hours after removal from the perisarc, coenosarc fragments have begun to redifferentiate (Goldin and

Barth, 1941), nevertheless, the differential exposure still determines the polarity of these regenerants. It is indicated, therefore, that the redifferentiation has not yet produced an irreversible determination of polarity.

The factors involved in the differential exposure appear to be oxygen and metabolic waste products. The diffusion rate of oxygen to the coenosarc tissue and of waste products away from the tissue is presumably lower inside the capillary than at the open end. The extent of this effect is indicated by the experiment of Barth (1938a), where inhibition was obtained even when the cut end of the *Tubularia* stem was flush with the end of the capillary. On this basis the coenosarc tissue is subjected to two gradients. There is a gradient of oxygen tension, with the high end at the exposed surface of the tissue, and a gradient of concentration of metabolic wastes, with the high end at the covered surface of the tissue.

TABLE I

The polarity developed by coenosarc fragments placed in the tips of glass capillaries T_1 and T_2 = time in hours after perisarc removal. T_1 = time at which the fragments were placed in the capillaries, T_2 = time at which the observations were made.

Description			Polarity of the regenerants			
Exp.	No. of fragments	T_1	T_2	Regeneration at exposed surface	No regeneration	Dead
1	43	24	96	28	7	8
2	20	39	135	7	2	11
3	18	43	88	4	8	6
4	16	48	163	10	3	3
5	48	48	192	30	12	6
Total	145			79	32	34

Either one, or a combination of both of these gradients might determine the new polarity relationships which develop during regeneration. If an oxygen gradient is the sole factor acting in the determination of the new polarity, it should be possible to obtain regeneration at any point on the coenosarc tissue by the application of a high oxygen tension at any localized point on the tissue, irrespective of the accumulation of waste products. Three experiments were designed in order to test the efficacy of oxygen in the presence of accumulated metabolic wastes.

In the first experiment dedifferentiated coenosarc fragments (26 hours after removal from the perisarc) were placed in the tips of capillaries so that half of the tissue surface was exposed to the sea water in the dish, while the other half was covered with the glass capillary. An oxygen bubble was then introduced into the capillary by means of a micropipette,

at a distance of from two to four millimeters from the coenosarc fragment. The size of the oxygen bubble was such as almost to fill the capillary.

The observations were made 96 hours after the coenosarc fragments were placed in the tips of the capillaries. Out of a total of 37 fragments treated in this way, 14 regenerated at the free end of the capillary, two regenerated in the direction of the oxygen bubble, 11 failed to regenerate, and ten died. Control coenosarc fragments, placed in the tips of the capillaries as in the previous experiment, with no oxygen bubble introduced into the capillary, all regenerated towards the free end of the capillary.

In the second experiment, dedifferentiated coenosarc fragments were placed in the center of glass capillaries containing sea water. An oxygen bubble was then introduced into one end of the capillary, and a nitrogen bubble into the other end of the capillary. The oxygen and nitrogen bubbles were of approximately the same length (8 mm.) and were placed about 2 mm. from the coenosarc fragment. Twenty coenosarc fragments (30 hours after removal from the perisarc) were treated in this way. After 120 hours none of the coenosarc fragments had regenerated. Seven out of ten untreated control coenosarc fragments, free in sea water, regenerated during the same period. It may be noted that the gas bubbles introduced into capillaries showed no appreciable loss in volume during the experiment.

In the final experiment, the coenosarc was removed from the perisarc of the distal half of ten millimeter stem segments, the empty perisarc being left intact. An oxygen bubble was then introduced into the empty half of the perisarc, and the stems were ligated at both cut ends. Untreated stem segments, as well as stem segments from which the coenosarc of the distal half was removed, but in which the ends were ligated without the introduction of an oxygen bubble, were kept as controls. All of the stems were kept in running filtered sea water at $19 \pm 1^\circ \text{C}$.

A total of six out of 60 experimentals regenerated hydranths after 96 hours, in the direction of the oxygen bubble. The oxygen bubbles themselves were reduced, but in no case completely dissipated during the experiment. None of the ligatured controls regenerated during the same period; whereas 29 out of 40 cut, but unligatured, stems regenerated. The experimentals which did regenerate apparently did so because of the increased availability of oxygen. Failure of ninety per cent of the stems to regenerate, despite the presence of the oxygen bubble, was probably due to the inhibition caused by the accumulation of waste products.

THE EXTENT OF ACID METABOLITE ACCUMULATION

This experiment was designed in order to determine the extent to which acid metabolites accumulate when regeneration is inhibited by capping. This could be accomplished by measuring the change in the pH of the sea water inside of the glass tubing. Clean seven millimeter glass tubing was cut into 24 millimeter lengths, and submerged in filtered sea water. Both stem segments and coenosarc fragments were used, one segment or one fragment being inserted into each tube. The ends of the tubing were then plugged with absorbent cotton in order to prevent the stem segments and coenosarc fragments from falling out. Measurements of pH were made with a Beckman pH meter, the water contained in five glass tubes being pooled for each reading. Coenosarc fragments and stem segments were kept in standing sea water as controls.

Twenty-five coenosarc fragments, and twenty-two stem segments were placed in the glass tubes. As in the capillaries, there was complete inhibition of regeneration. The average pH of the sea water in the glass tubes containing coenosarc fragments dropped from 7.96 to 7.11 after 96 hours. With stem segments, the average pH was lowered from 7.96 to 6.90 during the same time interval. Twenty-one out of 35 control fragments regenerated, the pH of the sea water changing from 7.96 to 7.86, while 15 out of 20 control stem segments regenerated with a pH change from 7.96 to 7.90. Thus, associated with the inhibition, there is a lowering of the pH of the sea water.

THE EFFECT OF INCREASED HYDROGEN ION CONCENTRATION
ON REGENERATION

The results of the previous experiments indicated that the accumulation of acid metabolites has an inhibitory effect on regeneration. If the inhibitory action of acid metabolites is due to the increased hydrogen ion concentration of the sea water, it should be possible to obtain inhibition by artificial acidification of the sea water. For an accurate analysis it is necessary to maintain the oxygen tension of the sea water samples at a constant level, while the acidity is varied. The general procedure for adjusting the sea water samples to the desired pH values was essentially the same as that used by Smith and Clowes (1924), and Whitaker (1937), the pH being regulated with acid, base, and buffer. An attempt was made to get a range of pH values from that of normal sea water (pH 8-8.2) down to pH 3. After the addition of acid, the sea water was aerated vigorously in order to re-equilibrate with the atmosphere. This was necessary, since the addition of acid liberates free carbon dioxide.

The pH measurements were made, as before, with a Beckman pH meter, and the dissolved oxygen was determined using the Winkler technique. Two series of experiments were performed.

In the first series of experiments the sea water was acidified to pH 3 by the addition of concentrated HCl. Air was then bubbled vigorously through the sea water for six hours, after which time successive measurements gave constant pH readings. Measured quantities of sea water (100 cc.) were then adjusted to a series of pH values by the addition of the following reagents: 0.2M NaOH; 0.6M NaHCO_3 ; 0.2M NaOH—0.6M NaHCO_3 ; and McIlvaine's buffer (Citric acid 0.1M—secondary sodium phosphate 0.2M). The sea water made up to the different pH values was poured into two-inch finger bowls placed in a constant temperature bath at $19 \pm 1^\circ \text{C}$. Stem segments taken from the region 5–15 mm. proximal to the hydranth were placed in each finger bowl and samples of sea water removed for determination of pH and dissolved oxygen. The stems were observed for regeneration when, by gross inspection, most of the control stems in normal sea water had formed primordia. The pH and the oxygen tension were determined again at the close of the experiment.

In a second series of experiments the sea water was acidified to the desired pH by the addition of concentrated hydrochloric acid. Air was then bubbled through the acidified sea water samples until constant pH readings were obtained. Stem segments were introduced into these sea water samples as in the previous experiment.

The results are summarized in Table II. As the hydrogen ion concentration of the sea water is increased, fewer stems regenerate during a given period. After 55 hours, 19 out of 20 stem segments regenerated at pH 8.08–8.04 (normal sea water), while only eight out of 20 stem segments regenerated at pH 7.05–7.04 (Table II, Exp. 3). The inhibitory effect was more pronounced as the pH was lowered, so that at approximately pH 6 no regeneration occurred. After 60 hours, four out of ten stems regenerated at pH 6.02–6.29, while at pH 5.93–6.08 there was complete inhibition (Table II, Exp. 1). The stems at the latter pH were kept in the experimental medium for a longer period (95 hours), at which time they still showed no signs of regeneration. As low as pH 6, the inhibitory effect is reversible, inhibited stems regenerating when returned to normal sea water. Below pH 6 the coenosarc tissue of many of the experimentals appeared to undergo gradual cytolysis and no recovery occurred. It may be noted that the oxygen concentration of the sea water samples was approximately the same for each experiment, and

that the concentration is in the range usually found in normal sea water. This rules out the possibility that inhibition was due to lack of oxygen.

DISCUSSION

Coenosarc fragments fail to regenerate when they are inserted into capillary glass tubing. A similar effect was demonstrated by Barth

TABLE II

The Effect of Increased Hydrogen Ion Concentration on the Regeneration of Tubularia Stem Segments

Medium	Initial pH	Final pH	Initial O ₂ cc./l.	Final O ₂ cc./l.	Number of regenerants
Experiment 1. Observed at 60 hours 10 stems at each hydrogen ion concentration					
Normal sea water	7.93	7.88	4.4	4.2	8
Acid S.W. + NaHCO ₃	7.89	7.94	4.0	4.1	7
Acid S.W. + NaOH-NaHCO ₃	7.87	7.42	4.4	4.1	7
Acid S.W. + NaOH	6.02	6.29	4.0	4.2	4
Acid S.W. + McIlvaine's	5.93	6.08	4.4	4.1	0
Acid S.W. + McIlvaine's	5.44	5.90	4.2	4.1	0
Experiment 2. Observed at 72 hours 10 stems at each hydrogen ion concentration					
Normal sea water	7.89	7.77	4.6	5.0	9
S.W. + HCl	6.77	7.23	4.6	4.9	7
S.W. + HCl	6.71	7.08	4.7	4.9	6
S.W. + HCl	6.57	7.19	4.6	4.8	6
S.W. + HCl	6.39	6.84	4.6	4.8	5
S.W. + HCl	4.88	5.70	4.8	4.5	0
S.W. + HCl	3.28	3.26	4.8	5.0	0
Experiment 3. Observed at 55 hours. 20 stems at each hydrogen ion concentration					
Normal sea water	8.08	8.04	4.6	4.6	19
S.W. + HCl	8.00	7.93	4.8	4.8	16
S.W. + HCl	7.91	7.74	4.7	4.7	14
S.W. + HCl	7.82	7.76	4.7	4.7	15
S.W. + HCl	7.62	7.56	4.7	4.7	11
S.W. + HCl	7.05	7.04	4.7	4.7	8

(1938a) using cut stems with perisarc, where covering of the cut end with glass tubing was sufficient to cause inhibition. The capillary walls decrease the diffusion rate and this might cause the oxygen tension of the sea water to fall below the level required for regeneration. In addition,

acid metabolites would accumulate in the sea water surrounding the tissues. Either one of these factors, or their combination, might cause the inhibition.

When the coenosarc fragments are placed in the tip of a capillary so that half of the tissue surface is covered by the capillary, while the other half is exposed to the sea water in the dish, regeneration always occurs at the exposed surface. Thus, the new polarity relationships developed by the homogeneous mass of tissue may be strictly determined as the result of a differential exposure to the environment. The differential exposure probably consists of two gradients, a gradient of oxygen tension, with the high end at the open end of the capillary, and a gradient of acid metabolite concentration, with the high end inside the capillary.

That oxygen plays a necessary role in regeneration has been clearly elucidated by other investigators. There is a threshold of oxygen tension below which regeneration will not occur, while above this threshold, the rate of regeneration is a function of the oxygen tension (Barth, 1938a). Miller (1937) obtained a reversal of polarity of *Tubularia* stem segments when the proximal cut end was bathed with oxygen and the distal end was bathed with nitrogen. Here, since the active bubbling of oxygen and nitrogen would remove waste products at the cut ends, the effect of oxygen was probably not vitiated by the inhibitory action of accumulated acid metabolites. The effectiveness of oxygen in regeneration is, however, limited by the inhibitory action of accumulated acid metabolites. This view is supported by the following evidences.

Most of the coenosarc fragments placed in the tips of capillaries regenerate in the direction of the exposed surface, despite the presence of a large oxygen bubble inside of the capillary. It may be assumed that the oxygen supply inside of the capillary was at least equal to that provided by normal sea water, so that regeneration should occur inside of the capillary. Since waste products presumably accumulate inside of the capillary, but are removed at the free end, it is indicated that the polarity of the regenerants was determined by the differential exposure to metabolic wastes. Thus, when there are no appreciable differences of oxygen tension at localized portions of the coenosarc fragments, a differential of the concentration of waste products may determine the polarity of the regenerants.

Coenosarc fragments inserted in glass capillaries do not regenerate when an oxygen bubble is placed on one side of the fragment and a nitrogen bubble on the other side. Failure to regenerate is probably due to the accumulation of acid metabolites, for again, sufficient oxygen was apparently available for regeneration to occur under normal conditions.

This interpretation is supported by the experiments of Rose and Rose (1941) in which it was demonstrated that regeneration is inhibited despite the presence of an oxygen bubble, when a cut end of a *Tubularia* stem is inserted into a tight-fitting cap. If the cap is loose-fitting, regeneration may occur.

Further evidence is offered by the experiments in which oxygen bubbles were introduced into stem segments. The coenosarc tissue was removed from the distal half of *Tubularia* stem segments. An oxygen bubble was then introduced into the empty half of the perisarc, and the cut ends were ligated. Ninety per cent of these stem segments failed to regenerate in running sea water, despite the presence of the large oxygen supply. This inhibition was probably caused by the accumulation of waste products. The stems which did regenerate, formed the hydranth at the distal end of the segment. Rose and Rose (1941) injected oxygen gas into the coelenteron of *Tubularia* stem segments and then ligated the ends. Twenty-one per cent of the stems regenerated when they were kept in running sea water, whereas none of the stems regenerated when they were kept in still water. Their results are understandable if waste products are removed more rapidly in an actively circulating medium.

The extent to which acid metabolites may accumulate as a result of capping is demonstrated by the change in pH of the sea water contained in glass tubing to which coenosarc fragments and stem segments had been added. In addition to inhibition of regeneration, there is a lowering of the pH of the sea water. With coenosarc fragments, the average pH of the sea water dropped from 7.96 to 7.11 after 96 hours, while with stem segments the average pH dropped from 7.96 to 6.90.

The acid metabolites probably cause inhibition by increasing the hydrogen ion concentration of the medium. The inhibitory action of increased hydrogen ion is demonstrated by the results of the experiments in which the pH of the sea water was regulated with acid, base, and buffer. The ability of *Tubularia* stem segments to regenerate is a function of the hydrogen ion concentration of the medium. At the oxygen tension of normal sea water, there is a range of pH in which regeneration is retarded, and a critical pH below which regeneration will not occur. The critical pH is probably related to the oxygen tension. Inhibition of stem regeneration in glass tubing occurred at a higher pH (pH 6.90) as compared with stems kept in open dishes (pH 5.93–6.08). This difference may be accounted for, if, in the glass tubing, a lowering of the oxygen tension of the sea water occurred concomitantly with the accumulation of acid metabolites. This would mean that the oxygen tension of the sea water required for regeneration is a function of the hydrogen

ion concentration. If this is true, it is suggested that the interrelationship of these factors in influencing *Tubularia* regeneration may be worked out in detail by studying regeneration in sea water at varying oxygen and hydrogen ion concentrations.

SUMMARY

Environmental factors effecting regeneration and the origin of polarity in regeneration were investigated. The experiments were performed using coenosarc fragments and stem segments of *Tubularia crocea*.

Regeneration was inhibited when coenosarc fragments and stem segments were inserted into glass tubing. Concomitant with the inhibition, there was a measurable drop in the pH of the sea water contained in the tubing.

Coenosarc fragments inserted into the tips of capillaries regenerated at the exposed surface of the fragments. The same polarization resulted in most cases, even when large oxygen bubbles were introduced into the capillaries.

Coenosarc fragments placed in capillaries, failed to regenerate, when an oxygen bubble was placed on one side of the fragments and a nitrogen bubble on the other side of the fragments.

When oxygen bubbles were introduced into the emptied distal portions of *Tubularia* stem segments, and the cut ends of the stems were ligated, only ten per cent of the stems regenerated.

All of these results support the view that the effectiveness of oxygen in regeneration may be limited by the inhibitory action of accumulated acid metabolites.

Acid metabolites probably cause inhibition by increasing the hydrogen ion concentration of the medium, for at the oxygen tension of normal sea water, there is a range of pH in which regeneration is retarded, and a critical pH below which regeneration will not occur.

LITERATURE CITED

- BARTH, L. G., 1938a. Oxygen as a controlling factor in the regeneration of *Tubularia*. *Physiol. Zoöl.*, **11**: 179-186.
—, 1938b. Quantitative studies of the factors governing the rate of regeneration in *Tubularia*. *Biol. Bull.*, **74**: 155-177.
—, 1940a. The relation between oxygen consumption and rate of regeneration. *Biol. Bull.*, **78**: 366-374.
—, 1940b. The process of regeneration in hydroids. *Biol. Rev.*, **15**: 405-420.
GOLDIN, A., AND L. G. BARTH, 1941. Regeneration of coenosarc fragments removed from the stem of *Tubularia crocea*. *Biol. Bull.*, **81**: 177-189.
KOMORI, S., 1933. Effect of pH on the regeneration polarity of the *Tubularian* stem. *Annot. Zool. Japon.*, **14**: 99-102.

- MILLER, J. A., 1937. Some effects of oxygen on polarity in *Tubularia crocea* (abstract). *Biol. Bull.*, **73**: 369.
- , 1939. Experiments on polarity determination in *Tubularia* regenerates. *Anat. Rec.*, **75**: Supplement, 38–39.
- ROSE, S. MERYL, AND F. C. ROSE, 1941. The role of a cut surface in *Tubularia* regeneration. *Physiol. Zool.*, **14**: 328–343.
- SMITH, HOMER W., AND G. H. A. CLOWES, 1924. The influence of hydrogen ion concentration on unfertilized *Arbacia*, *Asterias* and *Chaetopterus* eggs. *Biol. Bull.*, **47**: 304–321.
- WHITAKER, D. M., 1937. The effect of hydrogen ion concentration upon the induction of polarity in *Fucus* eggs. *Jour. Gen. Physiol.*, **20**: 491–500.
- ZWILLING, E., 1939. The effect of the removal of perisarc on regeneration in *Tubularia crocea*. *Biol. Bull.*, **76**: 90–103.